CARNIVOROUS PLANT NEWSLETTER

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Front Cover: Comparison of *Utricularia jamesoniana* flowers from 2000m (left) and 1000m (right). Photograph by Sebastian Vieira. See article on page 14.

Back Cover: *Drosera capillaris* 'Emerald's Envy' Photograph by William Joseph Clemens. Article on page 12.

Carnivorous Plant Newsletter is dedicated to spreading knowledge and news related to carnivorous plants. Reader contributions are essential for this mission to be successful. Do not hesitate to contact the editors with information about your plants, conservation projects, field trips, or noteworthy events. Contributors should review the "Instructions to Authors" printed in the March issue of each year. Advertisers should contact the editors.Views expressed in this publication are those of the authors, not the editorial staff.

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A New Species of *Pinguicula* (Lentibulariaceae) from Nuevo León, México

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Keywords: new taxa: *Pinguicula nivalis*, Mexico.

Received: 1 November 2004

Abstract

A new species of *Pinguicula* from México is described and illustrated: *Pinguicula nivalis*, a gypsophilous endemic from the southern part of the state Nuevo León. According to Casper's monograph (1966) its taxonomic position matches the criteria of subgenus *Temnoceras* Barnh. em. Casper, but the species does not fit into any of the sections of this subgenus. A new section (*Microphyllum*) is proposed to include *Pinguicula nivalis*, together with two other related species (*Pinguicula immaculata* Zamudio & Lux and *P. gracilis* Zamudio).

Introduction

In February 1994, while exploring the region of Zaragoza, Nuevo León, we found a new *Pinguicula* species (see Figure 1) resembling *P. immaculata*. The flower, however, had larger and broader corolla lobes. Upon further examination of preserved and living specimens other interesting features came to light, and convinced us the plant represented a hitherto unknown species. The specific epithet, referring to snow, was chosen because the flowering plants, with their white petals, resemble snowdrops against the gypsum soil.

Results

Pinguicula nivalis Luhrs & Lampard, *spec. nov.*

Type: (MEXICO): Nuevo León: distr. Zaragoza, gypsum hills between Carpinteria and Zaragoza, 1350-1400 m alt., 14/Feb/1994, *H. Luhrs & S. Lampard s.n.* (Holotype: IEB! (spirit)).

Herba perennis. Folia radicalia rosulata, biformia; rosula "hiemis" numerosa 13-19, crassa, anguste oblonga, 4-6 mm longa, 1.0-1.5 mm lata, apicem versus ad ? longitudinis ciliata, pilis multicellularibus capitatis; rosula "aestatis" 8-10, petiolata, petiolo 4-7 mm longo, 1-2 mm lato, apicem versus ad plus minusve 1/2 longitudinis ciliato, pilis multicellularibus capitatis, lamina ovata, apice obtusa vel subrotundata, margine involuta, superne glandulis sessilibus et glandulis stipitatis vestita, 4-9 mm longa, 4-8 mm lata. Hibernacula nulla. Pedicelli 1-2 erecti, atro purpurei, glabri, 20-45(70) mm alti, uniflori. Flores 11-16 mm longi (calcari incluso). Calyx bilabiatus, atro purpureus, extus glandulis stipitatis sparse obsitus; labium superum trilobum, lobis oblongo-ovalis, obtusis, 1.0-2.5 mm longis, 0.8-2.0 mm latis; labium inferum bilobum, lobis oblongo-ovalis, obtusis, 1-2 mm longis, 1 mm latis. Corolla bilabiata, alba, labio infero ad basi macula luteo-virescenti ornato; labium superum bilobum, lobis late obovatis vel obovato-rotundatis, apice undulatis, 3-7 mm longis, 3-6 mm latis; labium inferum trilobum, basin versus pilosus, pilis longis multicellularibus, pilis brevis multicellularibus capitatis et pilis multicellularibus cylindrico-subulatis in macula luteo-virescenti vestitis, lobis lateralibus obovatis vel late obovatis, apice undulatis, 4-8 mm longis, 3-6 mm latis, lobo medio paulo major, late obovato, apice undulato, emarginato, 5.0-12 mm longo, 5.0-12 mm lato. Tubus brevissimus, infundibuliformis, 1-2 mm longus, 2-3 mm latus, intus pilosus, pilis multicellularibus cylindrico-subulatis retro conversis, basin calcari versus in lineis triabus ordinatis, sine palato. Calcar cylindricum,

apice conicum obtusum, pallide purpureum, 2.5-5.0 mm longum, 0.5-1.0 mm latum, cum tubo angulum obtusum (± 90°) formans. Stamina 2, geniculata. Antheris ellipsoidalibus, purpureis, 0.5 mm longis, 0.9 mm latis. Ovarium subglobosum, atro purpureum, 0.5-1.0 mm longum, 2 mm latum, glandulis stipitatis parvulis obsitum. Stigma bilabiatum, rubro-purpureum, labio infero superiorem superanti, suborbiculato, fimbriato. Capsula et semina ignota. Florescentia I-II.

Perennial herb. Leaves rosulate, dimorphic; those of the winter rosette numbering 13-19, thick, narrowly oblong, 4-6 mm long, 1.0-1.5 mm wide, covered from the apex to half its length with long multicellular capitate hairs; leaves of the summer rosette numbering 8-10, petiolate, petiole 4-7 mm long, 1-2 mm wide, covered from the apex to approximately half its length with long multicellular capitate hairs, leaf blade ovate, obtuse or somewhat rounded at the apex, the margin involute, the surface covered with sessile and stalked glands, 4-9 mm long, 4-8 mm wide. Hibernacula absent. Scapes 1-2, erect, dark purple, glabrous, 20-45(70) mm tall, 1-flowered. Flowers (including the spur) 11-16 mm long. Calyx 2-lipped, dark purple, sparsely covered with short stalked glands; superior lip 3-lobed, the lobes oblong-oval, obtuse, 1.0-2.5 mm long, 0.8-2.0 mm wide; inferior lip 2-lobed, the lobes oblong-oval, obtuse, 1-2 mm long, 1 mm wide. Corolla 2-lipped, white, the base of the inferior lip marked with a lime-green patch; superior lip 2-lobed, the lobes broadly obovate or obovate-rotundate, the apex undulate, 3-7 mm long, 3-6 mm wide; inferior lip 3-lobed, covered with three types of multicellular non-glandular hairs towards the base, slender long hairs forming a broad border across the width of the inferior lip, short and broader yellow pigmented capitate hairs clothing the central part of the lime-green patch, and slightly longer white cylindrical-subulate hairs are arranged in two lateral rows at the base, the two lateral lobes obovate or broadly obovate, the apex undulate, 4-8 mm long, 3-6 mm wide, the middle lobe larger and overlapping upon the lateral lobes, broadly obovate, the apex undulate, emarginate, 5.0-12 mm long, 5.0-12 mm wide. Tube extremely short, funnel-shaped, 1-2 mm long, 2-3 mm wide, the inside covered with multicellular cylindrical-subulate hairs, pointing backwards and arranged in three rows towards the spur, palate absent. Spur cylindrical ending in a blunt conical tip, pale purple, 2.5-5.0 mm long, 0.5-1.0 mm wide, forming an obtuse angle (± 90°) with the tube. Stamens 2, the filaments abruptly bent. Anthers ellipsoidal, purple, 0.5 mm long, 0.9 mm wide. Ovary subglobular, dark purple, 0.5-1.0 mm long, 2 mm wide, covered with tiny stalked glands. Stigma 2-lipped, red-purple, lower lip much larger than the upper lip, suborbicular, the margin fimbriate. Capsule and seed unknown. Flowering January-February.

Additional collection examined: MÉXICO. Nuevo León: W. of Zaragoza, gypsum hillside, 1445 m alt., 16/Oct/1993, *Hinton et al. 23650* (TEX); sub nomine *P. aff. immaculata* Zamudio & Lux. Det.: H. Luhrs 1996.

Discussion

Pinguicula nivalis is endemic to a small area of gypsum hills in the vicinity of Zaragoza, southern Nuevo León, where it grows in gypsum soil amongst colonies of *Selaginella* and accompanied by *Agave* and *Hechtia* sp. at 1350-1450 m a.s.l. It flowers from the winter rosette only during January and February.

It is very closely related to *P. immaculata*, another gypsophilous endemic from central Nuevo León. Apart from minor differenees in both winter and summer rosettes, *P. nivalis* differs sufficiently from *P. immaculata* in the following characters noted in Table 1.

Pinguicula nivalis is also closely related to *P. gracilis*, a dweller of chalk rocks from northern Nuevo León, but from which it differs in the following characters noted in Table 2.

Although being different in many aspects, all three species show identical characteristic features that would place them in the same taxonomical position which conform more or less to the criteria of subgenus *Temnoceras* beeause of the following characteristics; the type of leaves and rosette, the two lipped corolla, the inferior corolla lip larger than the superior corolla lip, the middle inferior lobe emarginate and larger than the lateral inferior lobes, the funnel-shaped tube, and the cylindrical spur. However, they can not satisfactory be placed in any of the sections or subsections belonging to the mentioned subgenus (Zamudio, 1988, Zamudio & Lux, 1992). Therefore a new section (*Microphyllum*) is proposed to include *P. nivalis*, *P. immaculata* and *P.*

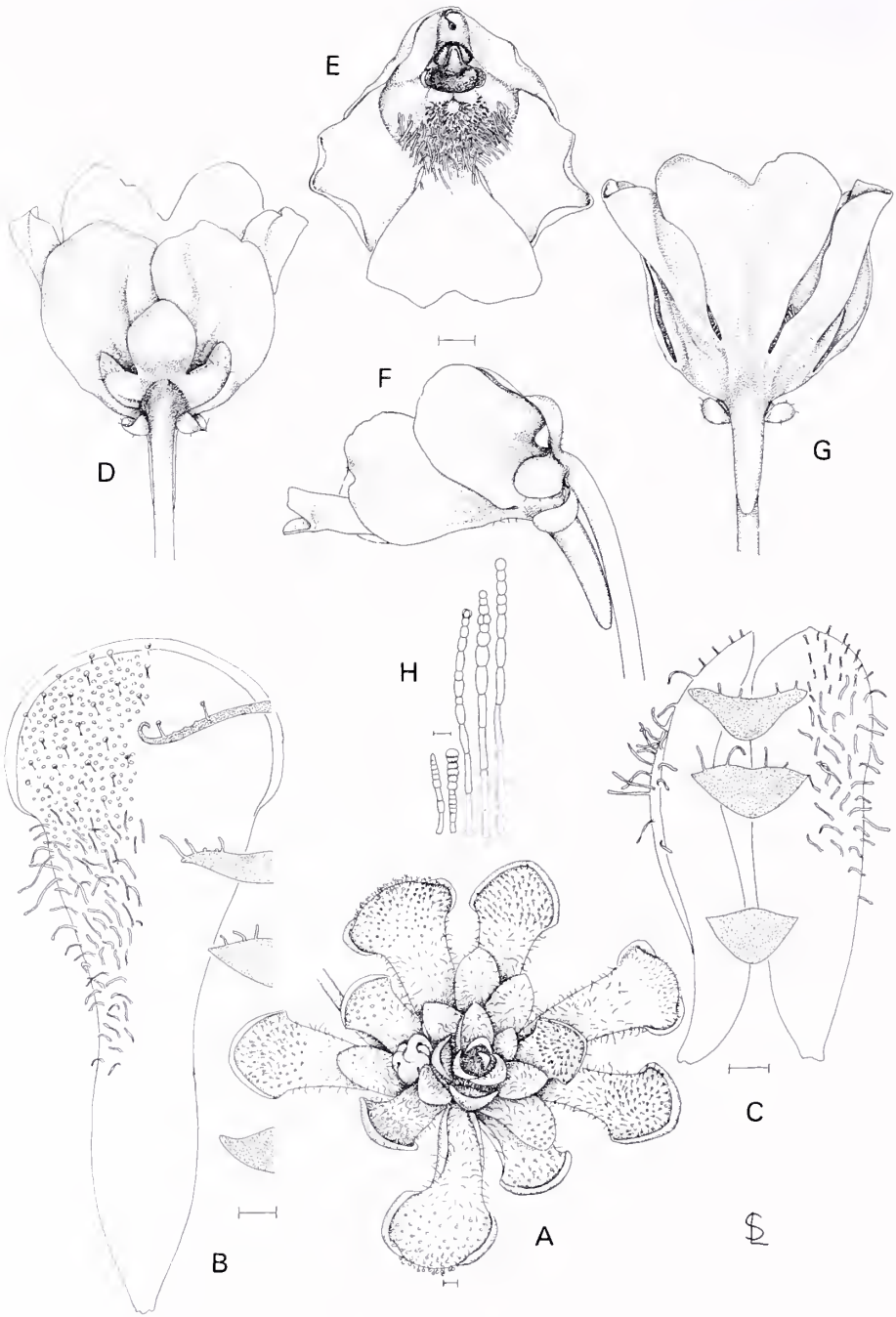

Figure 1: *Pinguicula nivalis*. A: habit, coming into winter rosette; B: summer leaf with transverse sections; C: winter leaf with transverse sections; D: calyx; E: corolla, not fully open; F: flower, lateral view; G: corolla tube and spur; H: hairs from right to left; 1-3 lower petal, 4 corolla tube entrance, 5 tube interior. Scale bars A-G 1 mm, H 0.1 mm. Illustration by Stan Lampard.

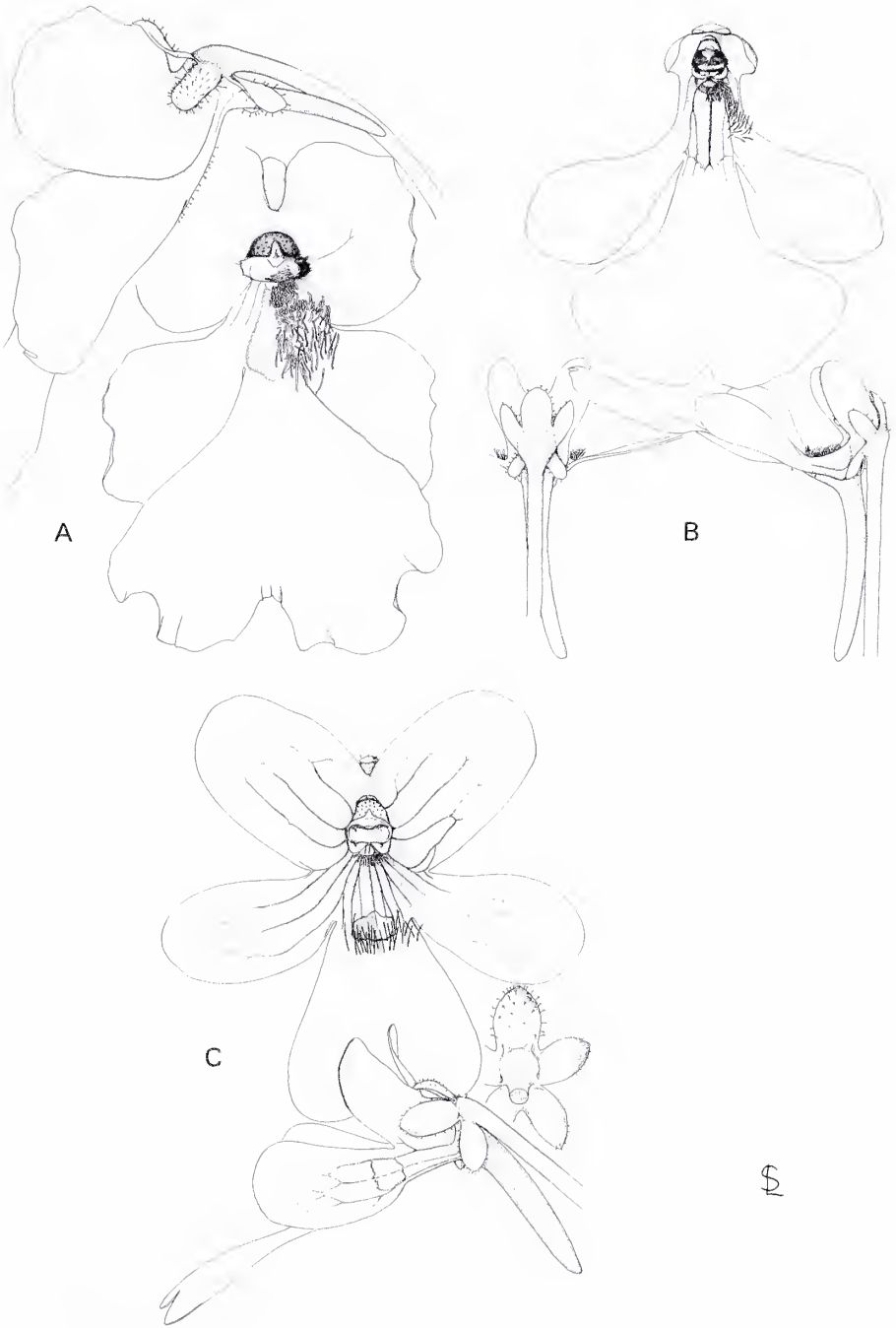

Figure 2: Corolla comparison of three related species of the new section *Microphyllum*. A: *Pinguicula nivalis*; frontal and lateral view; B: *P. immaculata*; frontal, lateral view and calyx; C: *P. gracilis*; frontal, lateral view and calyx. Illustration by Stan Lampard.

	P. nivalis	*P. immaculata*
Superior corolla lobes	Broadly obovate, 3-7 mm long/3-6 mm wide, margin undulate.	Oblong, 1-3 mm long/1.0-1.5 mm wide, margin entire.
Lateral inferior corolla lobes	Broadly obovate, 4-8 mm long/3-6 mm wide, margin undulate.	Obovate, 2.5-6.0 mm long/1.5-4.0 mm wide, margin entire.
Middle inferior corolla lobe	Larger and mostly overlapping upon the lateral lobes, broadly obovate, 5.0-12 mm long/5.0-12 mm wide, margin undulate, emarginate.	Larger than the lateral lobes, obovate, 5.0-10 mm long/4.0-10 mm wide, margin entire or undulate, emarginate.
Inferior corolla lip	The arrangement of three different types of multicellular non-glandular hairs covering the entire base.	The arrangement of two different types of multicellular non-glandular hairs covering the sides of the base, leaving a open space in the center.
Spur	Cylindrical, 2.5-5.0 mm long/0.5-1.0 mm wide, conical and blunt at the tip.	Cylindrical, 3.5-9.0 mm long/0.5-1.0 mm wide, blunt at the tip.

Table 1. Comparison of the floral features of *Pinguicula nivalis* and *P. immaculata*.

	P. nivalis	*P. gracilis*
Winter leaves	Narrowly oblong, 4-6 mm long/1.0-1.5 mm wide, covered from the apex to half its length with long multicellular capitate hairs.	Spatulate or oblanceolate, 3.0-12 mm long/1.5-4.0 mm wide, sparsely covered with long multicellular hairs.
Summer leaves	With a distinct petiole, 4-7 mm long/1-2 mm wide, the leaf blade ovate, 4-9 mm long/4-8 mm wide.	Obovate-spatulate, 8.0-16 mm long/4.0-10 mm wide.
Corolla	White.	White, radiated with purple veins in the throat.
Superior corolla lobes	Broadly obovate, 3-7 mm long/3-6 mm wide, margin undulate.	Oblong, 4-7 mm long/2.2-5.5 mm wide, margin entire.
Lateral inferior corolla lobes	Broadly obovate, 4-8 mm long/3-6 mm wide, margin undulate.	Obovate or oblong, 3.1-7.0 mm long/2.0-4.8 mm wide, margin entire.
Middle inferior corolla lobe	Larger and mostly overlapping upon the lateral lobes, broadly obovate, 5.0-12 mm long/5.0-12 mm wide, margin undulate, emarginate.	Larger than the lateral lobes, obovate-spatulate, 5.5-11.5 mm long/3.0-11 mm wide, margin entire, emarginate.
Inferior corolla lip	The arrangement of three different types of multicellular non-glandular hairs covering the entire base.	The arrangement of one type of multicellular non-glandular hairs covering the base of the middle inferior lobe.
Tube	Funnel-shaped, 1-2 mm long/2-3 mm wide.	Funnel-shaped, 2-4 mm long/3-6 mm wide.
Spur	Cylindrical, 2.5-5.0 mm long/0.5-1.0 mm wide, conical and blunt at the tip.	Cylindrical, 3.0-6.5 mm long/0.5-1.0 mm wide, blunt at the tip.

Table 2. Comparison of the main features of *Pinguicula nivalis* and *P. gracilis*.

gracilis (see Figure 2). The section's epithet refers to the small leaves of the three mentioned species.

Sectio ***Microphyllum*** Luhrs, *sect. nov.*

Folia biformia, rosulariter hibernantes. Corolla profunde bilabiata; labio infero supero plus minusve 2-3plo vel multo maiore; lobo intermedio labio infero lateralibus subduplo vel multo longiore, emarginato. Tubus brevissimus, palatum nullum. Calcar cylindricum, cum tubo angulum distinctum formans.

Leaves dimorphic, forming a winter rosette. Corolla deeply 2-lipped; the inferior corolla lip about 2-3 times the size or larger than the superior corolla lip; the middle inferior lobe emarginate and nearly twice the length or longer than the lateral lobes. Tube extremely short without a palate. Spur cylindrical, forming a distinct angle with the tube.

Type species: *P. immaculata* Zamudio & Lux.

Some elements in the criteria of subgenus *Temnoceras* do not exactly match with the three mentioned species, as well as the new section *Microphyllum*, e.g. the existence of a palate versus the lack of a palate, and the feature "distinctly broad conical" versus "funnel-shaped" in case of the tube. It might be questionable whether a more or less conformation rather than a exact one would be justified in the adding of a new member, while on the other hand is it hard to imagine that the proposal of a new subgenus for such minor characteristics would be a justifiable action. For that reason we believe some minor changes need to be made within the criteria of at least one subgenus (*Temnoceras*) and probably more than one section as well in a near future revision, in order to maintain their systematic value in the adding of a still growing number of newly described species.

Acknowledgements: We thank the anonymous referees for reviewing the manuscript, and Dr. Barry A. Rice and Dr. Jan Schlauer for their useful comments in the final preparations of this paper.

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Casper, S.J. 1963, Gedanken zur Gliederung der Gattung *Pinguicula* L. Bot. Jb. 82(3): 321-335.

Casper, S.J. 1966, Monographie der Gattung *Pinguicula* L. Bibl. Bot. 127/128: 209 pp.

Zamudio, S. 1988, Dos nuevas especies de *Pinguicula* (Lentibulariaceae) del centro y norte de México. Acta Bot. Mex. 3: 21-28.

Zamudio, S. and A. Lux. 1992, Una nueva especie gipsicola de *Pinguicula* (Lentibulariaceae), de Nuevo León, México. Acta Bot. Mex. 20: 39-44.

Zamudio, S. and J. Rzedowski. 1986, Tres especies nuevas de *Pinguicula* (Lentibulariaceae) de México. Phytologia 60(4): 255-265.

Looking Back: CPN 25 years ago

Allen Lowrie announced the discovery of white-flowered *Byblis gigantea* with a front-cover illustration in Carnivorous Plant Newsletter. He also derided a fanciful tale which, 25 years later, still circulates as "fact": "I read with amusement a paperback book on CP when I was in America in June, 1980. The author stated that *Byblis gigantea* in Australia catch rabbits and squirrels as their everyday prey. I can assure your readers this is not so. For a start, we don't have squirrels and secondly, the biggest prey *Byblis gigantea* can catch is small insects—generally mosquitos."

John Watkins also provided a profile on The Carnivorous Plant Society, which is of course the British society of renown. The Carnivorous Plant Society is still alive and active, with an excellent on-line discussion forum. See their web site at:
http://www.thecps.org.uk/

Barthlott, W., Porembski, S., Seine, R. & Theisen, I. 2004. Karnivoren, Ulmer, Stuttgart. 224 pp., text in German, 160 figs., many colour photographs, ISBN 3-8001-4144-2.

This is the first comprehensive book on carnivorous plants for readers with both a horticultural and scientific interest written by German authors. Chapters include habitats, biogeography and diversity, attraction and capture, digestion and utilization, prey, commensals, non-carnivorous plants capturing animals, evolution, cultivation, conservation, the individual families of carnivorous plants, bryophytes capturing animals, fungi capturing animals, alphabetical listing of carnivorous plants, glossary, literature, index, sources and societies. In general the book can be recommended to all German reading carnivorous plant enthusiasts, as it contains a good amount of interesting facts, views, and pictures, some of which not published before. Even apart from the text the book is worth a look for the photographs alone, some of which are really artistic. In one detail I do not agree with the authors who chose to call plants that capture and kill animals but that do not digest them "Praekarnivoren" (pre-carnivores). This term is not fortunate because it insinuates the predictability of a future evolutionary development, which would contradict the premises of evolutionary theory, and so far it still has to be proven if borderline cases like *Roridula* and some *Heliamphora* species are not in fact post-carnivorous. "Sub-carnivores" would have been a less problematic and more appropriate term. (JS)

Švarc, D. 2003 Masožravé rostliny, Sursum, Tišnov 184 pp., text in Czech, 251 colour photographs, many b/w line drawings, ISBN 80-7323-035-6.

Again a book from the remarkably active Czech carnivorous plant community. It can be recommended to cultivators (reading Czech) and to nature lovers and carnivorous plant book collectors for the many photographs. The reproduction quality of most pictures roughly corresponds to the comparatively low price of the book. The drawings are in a few cases a bit too "generic" to be of great use in identifying species. Some bromeliad species are treated as "experimentally proven" carnivores, while the cited experiments did in fact only show (endogenous) digestive enzymes to be lacking in their traps. (JS)

What is the closest non-carnivorous relative of Lentibulariaceae? Three publications finding more than four answers:

Jobson, R., Playford, J., Cameron, K.M. & Albert, V.A. 2003. Molecular Phylogenetics of Lentibulariaceae Inferred from Plastid *rps*16 Intron and *trn*L-F DNA Sequences: Implications for Character Evolution and Biogeography. Syst. Bot. 28: 157-171.

Müller, K., Borsch, T., Legendre, L., Porembski, S., Theisen, I. & Barthlott, W. 2004. Evolution of Carnivory in Lentibulariaceae and the Lamiales. Plant Biol. 6: 477-490.

Rahmanzadeh, R., Müller, K., Fischer, E., Bartels, D. & Borsch, T. 2005. The Linderniaceae and Gratiolaceae are Further Lineages Distinct from the Scrophulariaceae (Lamiales). Plant Biol. 7: 67-78.

While Jobson *et al.* (2003) is satisfied to place Lentibulariaceae as sister to a not well resolved Scrophulariales (Lamiales) containing *Antirrhinum*, *Linaria*, *Melampyrum*, *Digitalis*, *Veronica* (NB: all these genera are placed by the authors in "Scrophulariaceae", to which neither of them must belong if the family should exclude Lamiaceae), Gesneriaceae, Pedaliaceae, Acanthaceae, and Byblidaceae, Müller *et al.* (2004) offers a series of theories, depending on the method by which sequence homology is translated into a phylogenetic hypothesis: either Bignoniaceae (*Campsis* & *Kigelia* investigated for the *mat*K-*trn*K sequences) or Lamiaceae (*Lamium*) appear as the closest Lentibulariaceae relative. None of the other carnivorous or sub-carnivorous members of

Scrophulariales (Byblidaceae and Martyniaceae) is closer to Lentibulariaceae. Although Rahmanzadeh *et al.* (2005) pretends to focus on different lineages, a substantial portion of the phylogenetic trees is comprised of Lentibulariaceae, and—much to our surprise—yet another hypothesis is offered (based on the same *mat*K-*trn*K sequences): Acanthaceae (*Dipteracanthus*, *Thunbergia*, and *Avicennia*) as the closest Lentibulariaceae relative, followed by Martyniaceae (!). In a different analysis the already mentioned Bignoniaceae appear nearest to Lentibulariaceae again. Are we approaching a definitive solution yet? (JS)

Kameyama, Y., Toyama, M. & Ohara, M. (2005) Hybrid Origins and F_1 Dominance in the Free-Floating, Sterile Bladderwort, *Utricularia australis* f. *australis* (Lentibulariaceae). Am. J. Bot. 92: 469-476.

Using experimental crosses in cultivation, AFLP analysis, and chloroplast (*trn*T-*trn*L and *trn*Q-*trn*S) gene sequence comparison, the authors demonstrate that the widespread sterile *Utricularia australis* (forma *australis*) is derived from a hybrid between the two fertile taxa *U. macrorhiza* and what the authors prefer to call *U. australis* f. *tenuicaulis*. If *U. macrorhiza* is, however, considered specifically distinct from *U. australis*, the hybrid (or hybrid-derived taxon) must not be classified as an infraspecific entity of one of the parents (according to ICBN Art. H.5.1, the appropriate rank of a nothotaxon is that of the postulated or known parent taxa). Thus, it would be preferable to call the other parent *U. tenuicaulis* (the name is validly published but has just been treated as a synonym of *U. australis* in the past). The hybrid status of *U. australis* is not so surprising (it is sterile and shows hybrid vigour), the unusual part are the parents and their distribution. *U. tenuicaulis* is apparently confined to Japan, *U. macrorhiza* is widespread in North America (and rare in E Asia), while both are missing in most of the vast range (Old World except deserts) of *U. australis*, a situation somewhat similar to the other widespread hybridogenic taxon of carnivorous plants, viz. *Drosera anglica*, which is far more widespread than one of its parents, *D. linearis*. (JS)

Cheek, M., Jebb, M., Lee, C., Lamb, A. & Phillipps, A. 2003. *Nepenthes hurrelliana* (Nepenthaceae), a New Species of Pitcher Plant from Borneo. Sabah Parks Nature J. 6: 117-124.

The authors write "the appearance of this plant is overwhelmingly like that of a hybrid between these two species (rev. note: *N. veitchii* and *N. fusca*), which may in fact, be its origin." Nevertheless, the authors prefer not to treat it as a nothotaxon because it occurs in places where the parents have not been found. For other hybridogenic carnivorous taxa occurring outside the range of one or both of their parents cf. review above. (JS)

Forget anything?

If you need to renew your ICPS membership, do it this week, do it today. The year that your membership in the International Carnivorous Plant Society expires is printed on the mailing label for your issue of Carnivorous Plant Newsletter, right after your 4-digit member number. If it reads 2005, you must renew your membership to receive further issues. Timely membership renewal saves us money that could be spent printing more color illustrations in Carnivorous Plant Newsletter, conserving wild carnivorous plant populations, or devising other ways to help serve the ICPS membership better.

New Cultivars

Keywords: cultivar: *Drosera capillaris* 'Emerald's Envy'.

Drosera capillaris 'Emerald's Envy'.

Submitted: 4 December 2004

In the early 1970s I had infrequent opportunities to visit the Southeastern USA to seek populations of carnivorous plants and to observe them in their natural habitats. On one of these visits I discovered several habitats supporting diverse populations of carnivorous plants growing adjacent to the U.S. Naval Hospital at Pensacola, Florida. Whenever possible I returned to these sites and others to observe any changes human proximity was having on the carnivorous plant populations.

Later, in the 1980s I was able to visit the panhandle of Florida almost annually. This was due to my parents having relocated to Fort Walton Beach, near the Eglin Air Force Base where my father was employed at that time.

A corner lot almost directly across from the hospital was becoming very overgrown by shrubs and trees. Still, there was a great diversity of carnivorous plants struggling to survive in this environment, including *Sarracenia alata*, *Sarracenia purpurea*, *Sarracenia psittacina*, *Pinguicula planifolia*, and *Drosera capillaris*. It did not look like they would survive for much longer as they were already nearly buried by the overgrowth.

On my next visit I had a shocking surprise: There was no more overgrowth; in fact there was almost no vegetation at all. A few clumps of grass and scattered colonies of *Drosera capillaris* were the only vegetation of any kind that remained on the bare earth. From the equipment and materials at this site it appeared it might soon become an asphalt parking lot similar to many others that were already adjacent to it. This was in the early morning on Tuesday, 28 January 1986. In vain I carefully looked over the site to see if any of my other familiar carnivorous plants were still surviving. While doing so I noticed that among the colonies of dark red *Drosera capillaris* with pink flowers, one colony about a foot in diameter was different. Even though the plants in this colony were growing in full-sun, they were almost entirely green—the tentacles and gland tips were pale pink (see Back Cover). Further still, these plants had white flowers. This was quite a thrill.

I carefully collected only a few mature seed pods, some leaf cuttings from the larger plants, and a few seeds that were just starting to germinate while still inside their wet seedpods. In case I was mistaken about the impending doom of this site I did not wish to be responsible for the extirpation of this wonder from its natural home. I scoured the area for many more hours, but never found similar plants. I have never returned to this site.

About a year later I had managed to grow a very large colony of these plants while I was an undergraduate student of Horticulture at New Mexico State University. While the colony was thriving I harvested a very large quantity of seed and donated most of it to the ICPS Seed Bank, but I also kept some for myself. Subsequently I was unable to continue growing this plant (or for that matter, any carnivorous plants) for several years due to my travels. Eventually, once I was again able to resume my carnivorous plant horticulture hobby I discovered that all my seed of this variety had lost its viability. I sent out a request for seed from anyone who was still growing this material—my appreciation goes to "Sundew Matt" who provided me with viable seed of this interesting plant.

This plant, which I am naming *Drosera* 'Emerald's Envy', can be distinguished from other *Drosera capillaris* plants by the following features. The entire leaf petiole and blade exhibit light to medium green coloration, even when grown in strong artificial light or full sunlight. Other *Drosera capillaris* plants, if grown under low light levels, may appear similarly colored, so it is important to compare only plants grown in high light levels. Furthermore, the flowers of *Drosera* 'Emerald's Envy' are white—a somewhat unusual color (although white-flowered plants are

occasionally encountered in the wild). Mature plants range between 2.5 and 4.0 cm in diameter when grown in strong light, but may be even larger if grown in lower light levels.

Drosera 'Emerald's Envy' may be propagated by seed or vegetative means, but no matter how the plant is propagated, in order to retain the name *Drosera* 'Emerald's Envy', the progeny must exhibit the light green leaf color, white flower color, and maintain the form of the standard, even when grown under conditions of strong light (including full sun).

Drosera 'Emerald's Envy' is a tender perennial, persisting throughout the year. My current colony (planted among several pots) has been growing continuously since germinating in December of 2000.

I have chosen this cultivar name because the plant is predominantly green as are emeralds, and because I like gems almost as much as I enjoy growing and sharing carnivorous plants.

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Have you registered yet for the 2006 Conference of the ICPS? If you lost your registration form, you can do it online! (See the ICPS web site, www.carnivorousplants.org, or email conference organizers Doug Darnowski or Hong-Qi Li: ddarnows@ius.edu, hli@frostburg.edu.) Registration fees increase on April 1st, so register now! The conference is 1-5 June, and is planned to include presentation, vendors, artwork, and fieldtrips, and of course the opportunity to meet experts from around the globe!

Utricularia jamesoniana in cultivation

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Keywords: cultivation: *Utricularia jamesoniana*.

Utricularia jamesoniana belongs to the *Orchidioides* section of *Utricularia*, a group very popular and sought after by growers for their large orchid-like flowers. Among "Utric" fans there is a great desire to see the other species in this section become more common or successfully enter cultivation (see Figure 1). Despite this interest only one species, *U. alpina*, is commonly found in collections, although a few other species such as *U. asplundii* and *U. quelchii* are beginning to circulate.

In June of 2004, my coauthor (Sebastian Vieira) was kind enough to provide me (THW) with a number of *Utricularia jamesoniana* specimens (see Figure 2) from 'Represa el Peñol,' Antioquia, Colombia, 1900m a.s.l. *Utricularia jamesoniana* and *U. asplundii* are closely related and Taylor (1989) notes that plants intermediate in appearance between the two species can be found. However, Taylor believes the two species to be distinct and notes that the distinguishing characteristic between the two species is the length of the spur. As can be seen (Figure 5 and Front Cover) the spur from on these plants is significantly longer than the lower corolla lip, thereby confirming the identification of this plant as *U. jamesoniana*. By prior arrangement I distributed some of this material to other growers and also to the Atlanta Botanical Garden, in Georgia, USA. (Out of respect for the other growers' privacy we will not disclose their names.) Three plants were allotted to me and in this article I will discuss the techniques I have used to cultivate them.

The *Utricularia* species in the section *Orchidioides* are often, mistakenly, referred to as the "epiphytic *Utricularia*." This is inaccurate, because in truth most of the species from this section more often grow as terrestrials in habitat. My initial belief was that *U. jamesoniana* might be an exception to this, based upon Sebastian's description of 'Represa el Peñol.' Sebastian had also located a second population at 1000m a.s.l. where the plants grew as epiphytes on tree trunks on the sides of a small river (see Figures 3, 4). The habitat looked dry but the air was very humid because of the river's proximity. The plants grew on the sides of the tree trunks that faced toward the water. The plants and flowers of this lowland population appeared to be identical to the form provided to me. Sebastian's description of the habitat for these plants corroborated Taylor's (1989) description well, i.e. that the plant grows on:

> ...mossy tree trunks and branches in montane cloud forests or lowland rain forests from near sea level to 2500m altitude... From the information on collectors [sic] notes and my own personal experience in Panama and Ecuador this species almost invariably grows on trees from 1m to 5m or more above ground level.

I have observed significant stolon growth on all of my plants over the past six months. The nature of this growth initially led me to speculate that this plant is a true epiphyte. Despite vigorous growth the majority of the stolons produced by the plants run on or only just below the surface of the media; rarely do I find stolons descending deeper into the pots. By comparison I readily observe stolons growing out of the bottoms of all of my other *Orchidioides* pots, some potted up as recently as four months ago. In the case of one of the plants a stolon (10cm long) ran over the edge of the pot and is now creeping into the live *Sphagnum* in a neighboring pot of *U. reniformis*. This stolon was very thin and hair-like and stood 7cm in the air, branching every 1cm or so before toppling under its own weight. The portions that are in full contact with the live *Sphagnum* in the other pot are now visibly thicker and show signs of tuber and trap formation as

well as leaf growth. The portions of this stolon that are not in contact with any media remain hair-like. Similar runners (see Figure 1) are developing on my other plants though none are as substantial as the previously mentioned one. These stolons may be acting in a manner similar to the aerial stolons produced by plants like *U. nelumbifolia* and *U. humboldti,* allowing *U. jamesoniana* to scramble along and establish itself in mosses and detritus along branches. Given time, these plants may send runners throughout the surface of their pots and later develop into independent plants. My own observations along with those of Sebastian and Taylor led me to believe that *U. jamesoniana* is very likely a true epiphyte and that it rarely, if ever, grows terrestrially.

I planted the first of my three plants in a media that I use for all my *Orchidioides* section plants. This media consists of a 2-3cm layer of live long-fiber sphagnum moss overlaying an equal part mix of orchid bark, tree fern fiber and perlite or clay pellets. This combination of media has worked very well in the past and I believe that it closely mimics the conditions that an epiphyte would grow in. I potted the remaining two plants in different media based on information I had received from other growers on the media they prefer. The second medium was loosely packed long-fiber sphagnum moss while the final medium was an equal parts mix of peat and sand on top of which I placed a few strands of live *Sphagnum.* Sebastian has recently informed me that in July of 2004 his father found *U. jamesoniana* growing terrestrially on a rocky roadside bank at about 2000m elevation a.s.l. The soil profile consisted of a thin layer of earth covered with mosses and dripping water. These plants have particularly large, colourful flowers (see Front Cover and Figure 5). This finding confirms that there are indeed terrestrial forms of this species, or at least that the plant can grow terrestrially, and explains why the other media I had tried were ultimately successful. I have observed that plants in the long-fiber sphagnum and the plants in the peat and sand mix had a rapid decline after being kept very wet for ten days, so I do not recommend these media unless you can monitor the plants closely. Another soil mix configuration that seems to work well is to fill the pot half-way with fine grade pine bark, then top off the pot with a 50:50 mix of fine pine bark and long-fiber sphagnum, and add a final top dressing of live sphagnum. The plants establish quickly in this mix, seem more vigorous than in my other mixes, and the pot is less prone to being waterlogged.

Experience has taught me that *Orchidioides* plants must be grown in drained pots, as the presence of excess, stagnant water often results in the plants succumbing to rot. Orchid baskets and water lily-style net pots are excellent choices as they provide extra air circulation through the media. With extra attention to the moisture levels, regular pots can work fine as well. Given the diminutive size of *U. jamesoniana* I potted two of the plants in 10cm net pots and the third plant (that was in pure long-fiber sphagnum moss) in a standard 12cm square pot. While I am sure larger pots could be used I do not see that there would be any benefit from it. While I have yet to attempt it, I believe *U. jamesoniana* might also grow well mounted on a moss-covered slab of bark or branch.

Keep the media damp but not soaking wet. I top-water the pots and then leave them alone until the long-fiber sphagnum moss begins to look a little dry. I set the pots on Styrofoam blocks, thereby allowing the excess water to drain totally. By allowing water to drain into a tray, the local humidity level stays in the range of 50-90%, most often about 70%. I assume these plants can be conditioned to somewhat lower humidity levels but this should be done slowly as the leaves are very thin and would likely dry out rapidly.

Given the environment from which it originates, I believe that *U. jamesoniana* does not have a seasonal dormancy but is capable of going dormant when conditions become unfavorable (i.e. during drought.) My plants have not shown indications of dormancy but given the short period of time that I have been growing them I can not rule out the possibility that they have a dormant period. I assume a dormant state would be manifested by slowed growth, like that observed in the closely related *U. asplundii.* Should indications of dormancy occur, I would keep the media just damp enough to prevent the long-fiber sphagnum from becoming dry.

For lighting I using a pair of 120cm twin tube fluorescent fixtures hung 30cm above the plants. To provide a broad spectrum I have placed one "warm white" and one "Sunshine" bulb in each fixture. Some growers move their plants outdoor during good weather, and yet others grow theirs in sunlit greenhouses; in these situations it is probably best to protect the plant from

Figure 1: *Utricularia jamesoniana*, in cultivation in Georgia. The plant shown is potted in "epiphyte" mix. The "runner" stolon described in the text is quite obvious. Photograph by Travis Wyman.

Figure 2: A plant from El Peñol, 1900m. Photograph by Sebastian Vieira.

Figure 3: A cluster of epiphytic plants at 1000m. Photograph by Sebastian Vieira.

Figure 4: The habitat for plants at 1000m. Photograph by Sebastian Vieira.

Figure 5: A terrestrial plant from 2000m. Photograph by Sebastian Vieira.

direct sun. The leaves of this plant are very thin and delicate and I believe that prolonged direct sun light would likely burn them. When using sunlight, start with 30-50% shading but be ready to try more if the plant appears to need it.

It is important to maintain your plants in the appropriate temperature range. *Utricularia jamesoniana* grows under a broad range of conditions in the wild, from sea-level up to 2500m. The plants I have were collected at 1900m elevation, so I grow them with my highland *Nepenthes* and *Heliamphora*. My growing area for high elevation plants is in the crawlspace under my house. During the summer, daytime temperatures are generally at a maximum of 28°C though more often they range from 23-25°C. At night the temperature usually drops to about 18-20°C. During the winter the temperature range tends to be about 10 degrees lower.

I have not yet begun propagation efforts but I assume that these plants are, like most other *Utricularia*, propagated most easily via division. My technique for dividing *Orchidioides* plants is to remove a sizable clump from the mother plant during active growth and pot it in new media. I ensure that, for both the division and the parent plant, the humidity is high and the media only moist (if the media is too wet I find that the cutting and the mother plant tend to succumb to rot.) Occasionally the division or mother plant may lose many or all of their leaves. When this occurs treat the pot as if the plant were actively growing because in many cases the plant is simply establishing itself. I often see *U. jamesoniana* plants produce long runner stolons. If these are commonplace with *U. jamesoniana* then separation of one of these after it has formed leaves of its own might provide a more simple method of division.

Seed is also a probable means of propagation in cultivation but I would assume that, like the seed from related *Utricularia*, it must be very fresh or it will likely not be viable. I suggest sowing on milled *Sphagnum* and keeping it moist and under conditions of high humidity and good light. One of us (SV) attempted cross pollinating *U. jamesoniana* but obtained little seed—the resulting seed capsules looked almost empty, unlike other *Orchidioides* section species that produce large fruit full of seed. The seed was sent to another grower but was lost in transit, so we do not know if it was viable. This is discouraging but I (THW) have had similar occurrences with *U. alpina* and these results may just be a matter of technique. Time will tell.

In summary, this plant is fairly simple to grow, rivaling *U. alpina* in its ease. The leaves on all three of my plants have at least tripled in size and there are 2-4 times as many as when I first potted the plants. Because this plant has only recently entered cultivation, we currently do not have enough specimens to share extensively. We are working to propagate it to send to highly experienced growers and our close trading partners. In a few years it should be more commonly available, or so we hope. But for now it, unfortunately, remains difficult to obtain. Should we obtain extra plants, we will announce it via the internet community.

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Root anatomy of three carnivorous plant species

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Keywords: physiology: *Dionaea muscipula*, *Drosera adelae*, roots, *Sarracenia rubra*.

Introduction

The majority of terrestrial carnivorous plants grow in organic soils in bogs and fens, in which they encounter persistent unfavourable conditions. The soils are usually wet or waterlogged, mostly acid, usually poor in available mineral nutrients, and can contain phytotoxins (Juniper *et al.*, 1989). Generally, carnivory in most terrestrial plants may be considered as an adaptation to all of these stress factors (Adamec, 1997). Many carnivorous plant species take up the majority of N, P, K, Ca, and Mg for their growth by roots from mineral-poor wet soils but a weakly developed root system is a common characteristic for most species (Adamec, 1997, 2002). Roots are usually short and weakly branched. Nevertheless, knowledge of anatomical structure for carnivorous plant roots is very low and unsufficient (Guttenberg, 1968; Kutschera & Sobotik, 1992a,b). Recently, items of knowledge of carnivorous plant roots have been compiled by Adlassnig *et al.* (2005). Carnivorous plants grow with many non-carnivorous wetland plant species, mostly graminoids and cyperoids, which are characterized by a marked anatomical adaptation of their roots and rhizomes to soil anoxia which is based on development of voluminous air spaces in roots or rhizomes (Justin & Armstrong, 1987).

The aim of this paper is to present basic anatomical structure of roots of three terrestrial carnivorous plant species, *Dionaea muscipula*, *Drosera adelae*, and *Sarracenia rubra*, and to discuss whether the anatomical structure of their roots is related rather to carnivory or to an adaptation to soil anoxia.

Materials and Methods

All plant material was collected from a naturally lit greenhouse collection of carnivorous plants during June-July. The plants used were propagated vegetatively by dividing adult plants. They were grown in 10 × 10 × 10cm plastic pots in natural organic soils (Adamec, 2002). *Drosera adelae* F.Muell. (native to northeast Australia) was grown in a mixture of an acidic fen soil and perlite (approximately 6:1 ratio by volume), while *Dionaea muscipula* Ell. and *Sarracenia rubra* Walt. (both native to the southeast USA) were both grown in a mixture of conifer needle mould with vermiculite (approximately 4:1 ratio by volume; for the details see Adamec, 2002). The pots with the plants were placed in a 0.8m^2 white polypropylene container 0.4m high, filled with rainwater to a depth of 2-3cm.

One typical adventitious root was excised for each of three adult plants of each species. The length of the excised roots ranged within 3.8-6.5cm in *Drosera adelae*, 3.5-4.0cm in *Dionaea muscipula*, and 7.2-11.0cm in *Sarracenia rubra*. The roots were shaken thoroughly in tap water to remove soil particles. They were fixed with a 70% FAA solution[1]. Three-mm long segments of root tips, middle parts, and bases were embedded into paraffin. Ten-µm thick sections of root tips, and 15-µm sections of the other root segments, were cut using a microtome. The preparations were stained by 0.1% Alcian Blue (Alcianblau 8 GS, Fluka, FRG) in 3% acetic acid for 2 hours. Then they were stained by 0.1% Safranine (Safranine T, Fluka) in citrate-phosphate buffer (pH 4.0) for approximately 16 hours. Parallel preparations were tested for lignin by histochemical staining by 1% Phloroglucinol (Phloroglucinol, Fluka) in ethanol with HCl. All preparations were mounted to Solacryle (Solakryl, Synthesia, Kolín, Czech Republic). The proportion of air spaces to root cross-section area was estimated by scanning the photographs of cross-sections in which the air spaces had been blackened by hand. The proportion of central cylinder to the total root cross-section area was calculated from diameters of central cylinder and the root.

[1]A mixture of 5 ml 40% formaldehyde, 90 ml 70% ethanol, and 5 ml glacial acetic acid.

The Safranine stains provided unsatisfactory results. This method stains lignified xylem elements, and since parallel stains prepared using Phloroglucinol+HCl (a classical test for lignin) also demonstrated only weak staining, we conclude that the xylem elements in carnivorous plant roots contain a very low content of lignin. However, additional tests (Mäule reaction, aniline sulphate test) should be used to support this conclusion.

The anatomical structure in differentiated middle parts of carnivorous plant roots was the same as that in basal root parts. Root hairs were present in all three species, but the occurrence of root hairs was variable among the species and single roots. On some images, root hairs were not visible (see Figures 1-4). Generally, the anatomical structures of Droseraceae roots (e.g. *Drosera adelae, Dionaea muscipula*; Figures 1, 2) were similar, and differed from that of *S. rubra* (see Figure 3). Roots of *Drosera adelae* and *Dionaea muscipula* (middle and basal parts) were covered with impregnated one-layer-celled rhizodermis which was only partly kept in *Dionaea*. Exodermis (external layer of cortical cells below rhizodermis with suberinized cell walls having a protective function) was developed only in *D. adelae* and *S. rubra* roots (Fig. 1, 3), but not in *Dionaea* roots (Fig. 2). This finding is in harmony with Guttenberg (1968) and Adlassnig *et al.* (2005). Cortex in roots of both Droseraceae species was relatively thin and contained smaller and larger intercellular spaces, but no voluminous air spaces. Natively colored (brown), thin-walled endodermis was also impregnated and, judging from its stainability by Safranine, it was lignified. Casparian strips (*i.e.*, impregnated parts of radial cell walls) were not detected clearly in the endodermis in roots of either zone. A relatively thick central cylindrical zone contained radially arranged vascular tissues, the phloem and xylem. In *Drosera adelae*, two phloem poles occurred atypically also in central part of the central cylinder (see Figure 4). While this arrangement of phloem poles is rare, may occur in Droseraceae roots (Guttenberg, 1968; A. Lux, pers. commun.).

The middle and basal parts of *S. rubra* roots were covered with an intensively impregnated thin rhizodermis (see Figure 3). In contrast with the cortex of the Droseraceae plants, the cortex in *S. rubra*

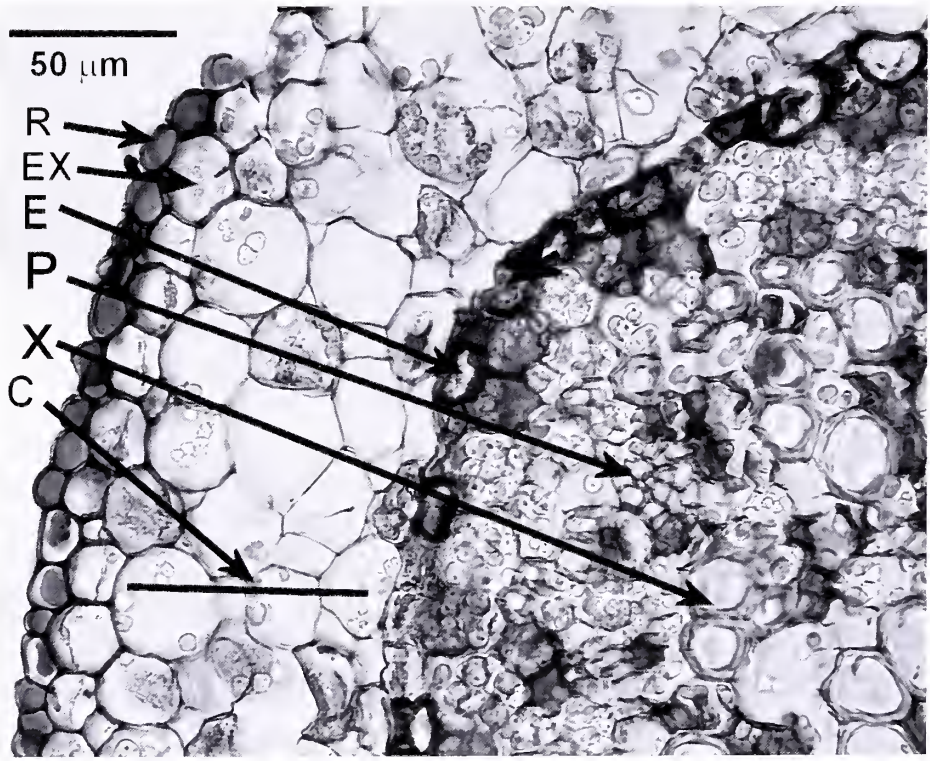

Figure 1: Cross-section through middle part of *Drosera adelae* root. The dark structures were stained by Safranine and Alcian Blue. R, rhizodermis; EX, exodermis; C, cortex; E, endodermis; P, phloem; X, xylem.

roots was distinctly subdivided into two zones. The external hypodermal zone consisted of three layers of small, natively colored (brown) cells, while the internal acrenchymatous zone was formed by a column-shaped cell arrangement around large air spaces. In some preparations, Casparian strips were present in the non-impregnated endodermis. The central cylinder contained a great proportion of sclerenchymatous tissues. Xylem poles were more distinct than phloem ones. Differentiated parts of roots of all three carnivorous plant species contained starch grains. The greatest starch content occurred in *Drosera adelae* roots (see Figure 1).

Generally, this study has revealed a considerable similarity of the root anatomy of carnivorous plants with that of other wetland non-carnivorous plants, especially dicot species (*e.g.*, Justin & Armstrong, 1987), and the degree of anatomical adaptation of carnivorous plant roots to soil anoxia may be discussed. The proportion of intercellular and air spaces within the differentiated roots could amount to approximately 5-10% of the total root cross section area in *Drosera adelae* and *Dionaea muscipula* (see Figures 1, 2), while approximately 20% in *S. rubra* (see Figure 3). These values are comparable with those of root porosity, estimated using a pycnometric method, reported by Justin & Armstrong (1987) for 42 wetland dicot and monocot plant species (usually to within 5-45%). Thus, carnivorous plants lie near a lower limit of root porosity in wetland plants. However, when information on the structure of carnivorous plant roots is combined with recent results on measurements of radial oxygen loss from carnivorous plant roots to anoxic medium (Adamec, 2005) two different strategies of oxygen economy within carnivorous plant roots may be suggested. Roots of Droseraceae with a low proportion of air spaces, prevent against radial losses of oxygen by an impregnated, impermeable rhizodermis or exodermis, and are able to conduct oxygen up to root tips. Meanwhile, in *Sarracenia* roots which have a greater proportion of air spaces, longitudinal diffusive oxygen fluxes are much greater, but a considerable part of oxygen leaks radially from the roots. As carnivorous plant roots grow under permanently anoxic soil conditions it is possible to conclude that roots of carnivorous plants are well adapted to living in anoxic soils and the aeration mechanism is sufficient to supply the whole roots with oxygen until the shoots are flooded.

Carnivorous plant roots do differ considerably from roots of wetland non-carnivorous plants in their

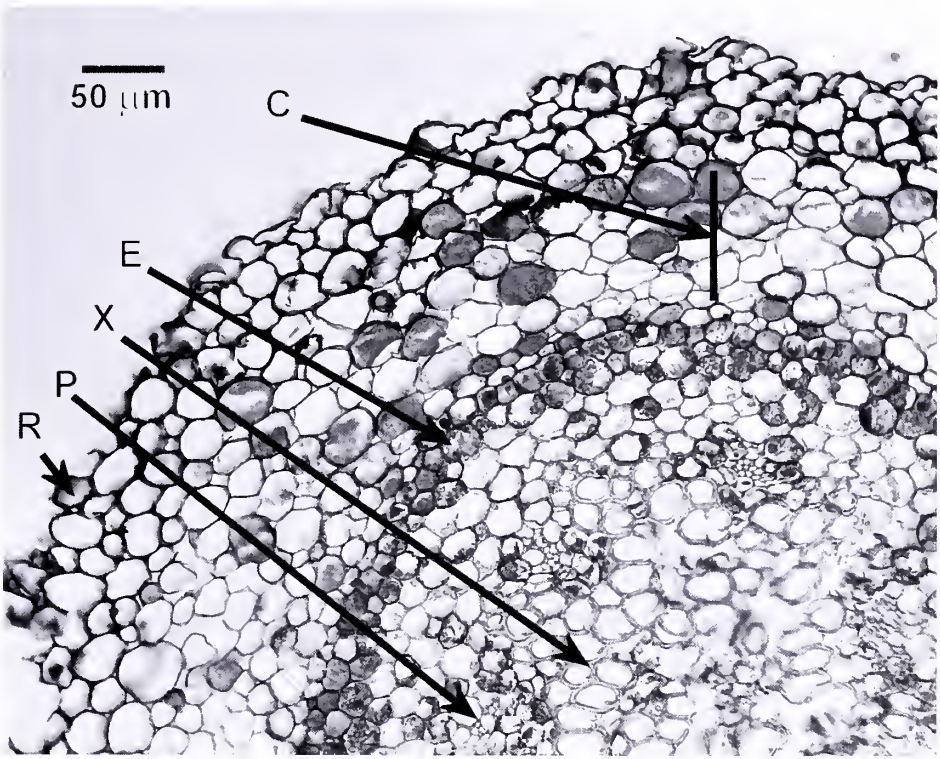

Figure 2: Cross-section through basal part of *Dionaea muscipula* root. R, rhizodermis; EX, exodermis; C, cortex; E, endodermis; P, phloem; X, xylem.

great proportion of central cylinder to the total root cross-section area. While the proportion in differentiated roots of several wetland non-carnivorous plant species was mostly within 3-8% and only exceptionally 34% (Justin & Armstrong, 1987), the proportion was much greater in all three carnivorous plant species investigated, i.e 46-48% in *Drosera adelae*, 34-38% in *Dionaea muscipula*, and 20-25% in *S. rubra* (calculated from the diameters of cross-sections and central cylinders). Such a great proportion of central cylinder with vascular bundles in carnivorous plant roots confirms their important role for pumping mineral nutrients and water to shoots. Moreover, on the basis of a recent ecophysiological study (Adamec, 2005), carnivorous plant roots appear to be physiologically very active per unit biomass, in spite of their relatively weak proportion. Since the prevailing amount of mineral nutrients in carnivorous plants is gained by roots, the activity of which is greatly stimulated by foliar nutrient absorption from prey (Adamec, 1997, 2002), the role of roots is crucial also for carnivory.

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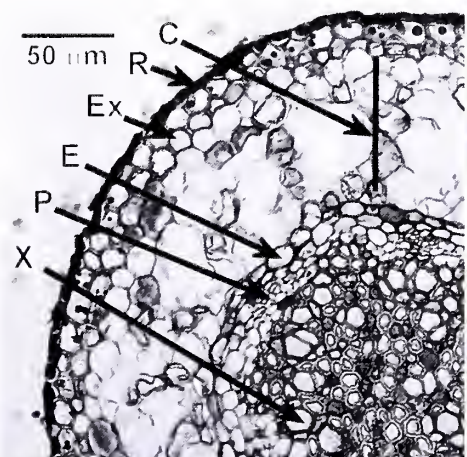

Figure 3: Cross-section through middle part of *Sarracenia rubra* root. R, rhizodermis; EX, exodermis; C, cortex; E, endodermis; P, phloem; X, xylem.

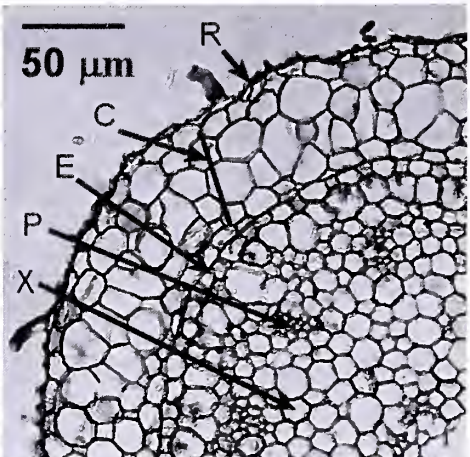

Figure 4: Cross-section through apical part (3 mm behind apex) of *Drosera adelae* root. R, rhizodermis; C, cortex; E, endodermis; P, phloem; X, xylem. All photographs by P. Kohout.

Tropical Pitcher Plant (*Nepenthes*: Nepenthaceae) Pollen Germinability and Storage: Conservation Implications

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Keywords: conservation: *Nepenthes* – cultivation: *Nepenthes*, pollination.

Introduction

Numerous *Nepenthes* species are at risk of extinction in the wild, with a dozen being on the IUCN Red List as "Critically Endangered" or "Endangered" (von Arx, *et al.* 2001; IUCN, 2003). The principal threats to wild *Nepenthes* are habitat destruction and in some cases the removal of plants by collectors (Clarke, 2001). Therefore, *ex situ* conservation measures should be implemented. Being able to quickly determine the viability of pollen and to store it until a female plant is blooming (*Nepenthes* are dioecious) will greatly enhance the chances of obtaining seeds and the next generation of plants.

In order to better understand options available for *ex situ* production of seeds, studies were undertaken to better define methods for collection and viability testing of pollen of *Nepenthes ampullaria, N. tentaculata,* and *N. ventricosa.* Methods for storage of pollen of a model species *N. ventricosa* were compared. Although the results discussed are preliminary, they give us a glimpse of what is possible and some recommendations for prolonged storage are made.

Beyond the pollen collection, storage, and pollen/stigma interactions; matters that concern pollen vigor and ultimate survival of a species in a maintenance program are issues of genetics and environment of the parent plants. Haphazardly collecting, storing, and placing pollen in an *ex situ* conservation program will ultimately result in a waste of time and materials with the possible extinction of the targeted plant species. Storage of pollen for prolonged periods (decades) seems feasible and should be started while pollen of the maximal number of individual plants are still available.

Methods

The hanging drop method involves the germination of pollen grains in a table sugar (sucrose) solution, as described for orchid pollen by Light & MacConaill (1996): pollen grains are placed on a hanging droplet of sugar solution suspended from the lid of an enclosed transparent surface such as a Petri dish. The pollen germination can be observed with a microscope from above. No wetting agent is used and many of the grains will remain on the lower surface of the hanging drop.

Fresh dilutions should be used because microorganisms rapidly populate sugar solutions retained at room temperatures. Alternately, aliquots may be prepared and frozen for later use.

Experimentation may be required to determine the correct droplet size to prevent drops from running when the lid is inverted, or evaporating before tube growth can be observed. Humidity within the closed Petri dish is maintained by adding water to the bottom of the dish. Several replicates of each test should be used so that failures due to bacterial/fungal contamination or drops falling during lid placement will not require replication of a test. The area where the dishes are placed should be of constant temperature, or water will condense on the lid of the Petri dish diluting the sugar solution.

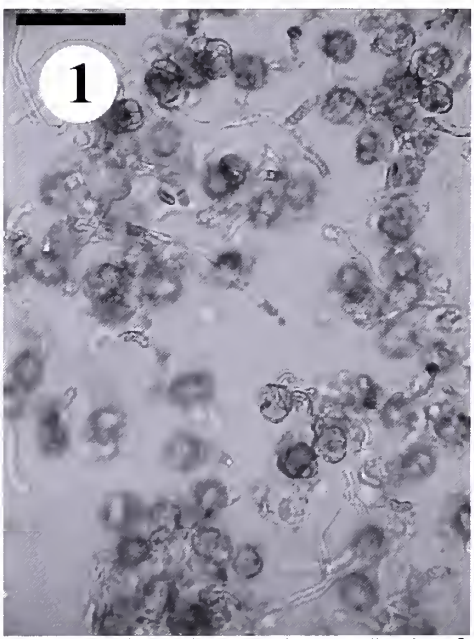
Although the pollen and tubes can be detected at 40X, the morphology is difficult to discern and any contaminating fungal bodies can be mistaken for tubes. Transmitted light microscopy with a compound microscope at 100X is ideal for rapid scanning of the Petri dishes.

Pollen viability means the ability of pollen grains to germinate on the stigma of a receptive flower, resulting in pollination and production of germinable seed (Dafni & Firmage, 2000; Firmage & Dafni, 2001, and citations therein). Unfortunately, measuring viability by seed set in *Nepenthes* is difficult and time consuming. The development of the fruit capsule generally takes 3-6 months (Clarke, 1997, 2001) and germination of the seeds can often take several additional months. Most indirect methods to assess pollen viability rely on stains which are reported to only dye living tissue. Several of these tests are not reproducible whereas others have been demonstrated to stain dead pollen. Further, many of the chemicals needed for these procedures could potentially be difficult to obtain in some parts of the world. This latter potential problem is especially important when such methods are needed for testing pollen in remote situations. A particularly promising method involves the germination of pollen grains in a table sugar (sucrose) solution.

Pollen germination (production of pollen tubes) occurs at room temperatures often within 12 hours. Longer periods are needed for some pollen but anything beyond 36 hours suggests problems with the pollen. The first stage of germination is the production of finger-like tubes from the pollen grain. The tube will elongate in a few hours and become tangled in nearby pollen tubes. Germinated grains with pollen tubes are depicted in Figures 1-4. Early stages of fungal growth appear similar to pollen tubes but fungi have multi-branched tips (Figure 5).

The hanging drop method only requires a very minute amount of pollen to test germinability, but the pollen should be well mixed so a representative sample is used. Enough pollen will adhere to the point of a pin or probe for a drop of sugar solution. If germination percentages are to be recorded, the number of grains per drop should be reduced and the grains germinating should be counted before they become tangled (Figures 1-2 are examples of over-grown/crowded preparations). In addition to the percent of grains germinating, any deformed grains or pollen tubes should be noted as these could signal future pollination problems.

Pollen of *Nepenthes ventricosa* germinated in 0.4-0.9 M sucrose, but 0.6 M solution was optimal. *Nepenthes ampullaria* germinated in 0.6 M whereas *N. tentaculata* did not; sufficient pollen of



Figures 1: *Nepenthes ventricosa* pollen in 0.6 M sugar solution, harvested from freshly opened flowers where nectar and pollen are actively being produced. Scale bar = 0.1 mm.

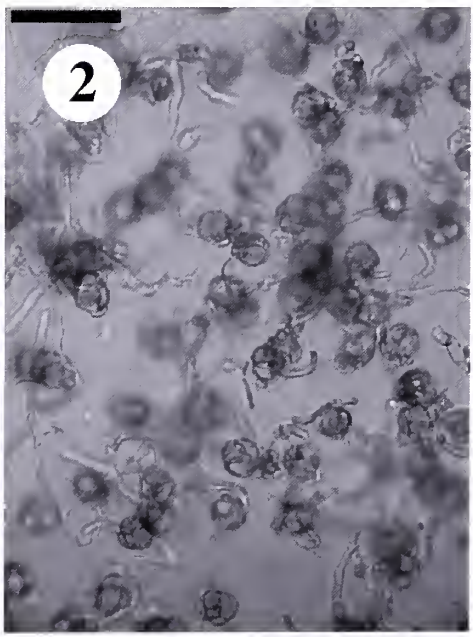


Figure 2: *Nepenthes ventricosa* pollen in 0.6 M sugar solution, harvested from flowers that have opened a few days previously. Scale bar = 0.1 mm.

these two species was unavailable for testing at other sucrose concentrations. (The *N. tentaculata* pollen was at least partially viable as a portion was successfully used to pollinate another plant, as described below.) When testing other species it is suggested to use a series of sucrose dilutions (0.1 to 0.9 M) to determine which is optimal for that species.

While germinating the *Nepenthes ampullaria* and *N. ventricosa* pollen, illumination was held constant at about 30000 lux (3000 fc; from a 75 watt incandescent light at 25 cm). When pollen was placed in 0.6 M sugar solution (an optimal dilution) but kept in the dark for 48 hours, no tubes were seen. The dish was placed under illumination (40 watt light at 25 cm), and tubes were observable within an additional 48 hours. While the pollen of *Nepenthes ampullaria* and N. *ventricosa* would not germinate in dark or low light conditions, initial tests for other species should be run in both dark and light situations.

Diurnal Variation in Pollen Viability

Male and female *Nepenthes* flowers are small but are easily distinguished by close examination of the external morphology. Illustrations of these differences are available in numerous publications, including those by Clarke (1997: Figure 6), Kurata (1976: Figure 4, plate 25), and Pietropaolo & Pietropaolo (1986: Figures 3-12). Flowers of both sexes lack petals but have four, slightly cup-shaped sepals with numerous large nectar glands. Female flowers have the much fatter ovary (often as wide as long) with the white to yellowish to pink colored stigma on top. The flowers open slowly and remain open with the sepals reflexing over time until they wither and dry.

Although *Nepenthes* hybrids have been artificially created in hothouses since the mid-1800s, pollination has been little studied (but see Chua, cited in Clarke, 2001; Frazier, 2000; Frazier, in Clarke, 2001; Kato, 1993; Kaul, 1982; Moran, 1993). These researchers have found a number of potential pollinators, such as flies, moths, beetles, wasps, etc., and that some species may be pollinated during the day, while others may be pollinated at night or during both times. With the large habitat diversity in the genus *Nepenthes*, pollen production and viability data obtained from one or a few species should not be applied to all members of the genus. Further testing will be needed to better define conditions for pollination in each potentially endangered *Nepenthes* species.

Flowering of *N. ventricosa* was studied in a greenhouse. This species produced nectar in the late evening and night which would dry during the early hours of daylight. It had a very strong and distinctive foul smell during the night. The nectar is attractive to Dolichoderinae ants and fruitflies which were seen on the *Nepenthes* inflorescences in the same greenhouse. This indicates that female *Nepenthes* flowers should be isolated if any male *Nepenthes* are concurrently flowering in the same greenhouse.

Pollen was collected from *N. ventricosa* for two days by tapping the entire inflorescence over a piece of wax paper and then rolling the end of the paper to form a funnel so that the pollen could be shaken into a plastic microtube (cryotubes suitable for storage in liquid nitrogen). Pollen collected at the first sampling of several different inflorescences revealed large numbers of non-viable pollen grains. This suggests that pollen should be removed prior to any collection to verify that older pollen was not left on the inflorescence. This is especially important in a greenhouse where insect pollinators and high winds are not usually present.

Pollen was collected at the end of each period: 8-11 AM, 11 AM-12:30 PM, 12:30-3.30 PM, 3:30-8 PM, 8-10 PM, 10 PM-12 AM, 12 AM-8 AM. Viability of pollen collected during the night (10 PM-8 AM) was excellent with virtually 100% of the grains forming tubes. In significant contrast, only 2.6-5.0% of the grains collected during the day (8 AM to 10 PM) showed tubes from pollen.

This pilot experiment demonstrated in this species that peak periods of odor and nectar production correspond with the release of viable pollen. It also demonstrated that most pollen collected during the daylight hours was either dead or in some form of stasis such that it would not produce pollen tubes under the standard conditions listed earlier. This reduced vigor has not been reported for *Nepenthes*, even among hybridizers. The reason this has not been noted may be because even 5% viable pollen will result in sufficient seed set if many thousands of grains are transferred during pollination. It is interesting to note that pollen of this species requires light in order to germinate, but peak pollen viability is apparently during the night.

Pollen of some plants (including the carnivorous plant genus *Pinguicula*; pers. com. M. Studnicka 2004) is completely developed in very young anthers, prior to dehiscence. So, viable pollen waits for some time to be released in these plants. An interesting follow-up study would be to determine if mature pollen enclosed in undehisced anthers are viable at all points in the diurnal cycle.

Storage

In the early 1800s *Nepenthes* hybridizers stored pollen at room temperatures and were only able to keep it viable for a few days. More recently individuals have attempted storage by keeping pollen at reduced temperatures. Slack (1986) stated that pollen could be stored in the main compartment of the home refrigerator for "some" weeks. Pietropaolo & Pietropaolo (1986) stated that pollen stored well under refrigeration (3°C/38°F), surviving for "at least one year in all species of which we are aware." They also provided a methodology for drying and freezing pollen for *Gloxinia*, but stated at that time no one had tried it on any carnivorous plants. D'Amato (1998) apparently was the first to report that pollen of such species could be stored for up to one year in foil packets in the home freezer. Clarke (2001) also stated that pollen can be stored for "several weeks in a freezer before use." Unfortunately, none of these authors provided precise details on how they treated the pollen before freezing. Presumably, all their results are based upon production of seeds as a measure of viability because they mention no other means of testing.

Different storage methods were investigated, to learn more about the factors important in reducing stored *Nepenthes* pollen viability. *Nepenthes ventricosa* pollen collected during the night was stored for differing durations in the dark at: room temperature (20-22°C/68-72°F), home refrigerator (about 3-4°C/37-39°F), and home freezer (about -18°C/0°F). Tests were performed under four sets of conditions: open vial in sealed larger jar with desiccant (approaching 0%R.H.), sealed vial with pollen in water, sealed vial dried to room humidity (approximately 40%R.H.), open vial in sealed larger jar with a slurry of calcium chloride (30%R.H). Room temperature stored pollen was air dried in an atmosphere of approximately 40-50%R.H.

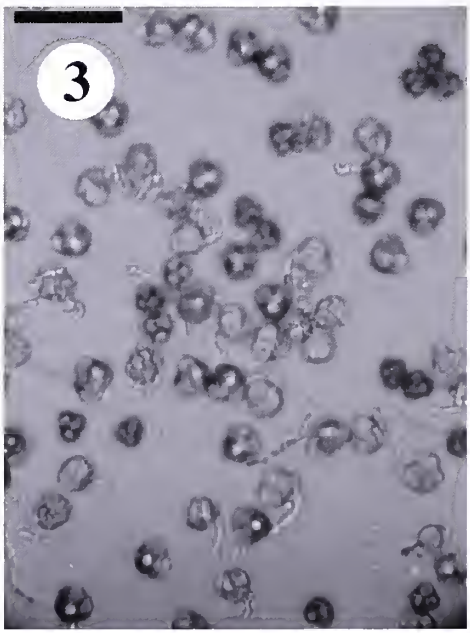


Figure 3: *Nepenthes ventricosa* pollen in 0.6 M sugar solution, harvested from old flowers lacking nectar production, and with sepals that are reflexed. Scale bar = 0.1 mm.

After three days of storage, germination of pollen stored at room temperature was drastically reduced. By the 8th day only 10 pollen tubes could be located out of thousands of grains tested. Pollen stored in water for one week showed very low germination whereas the drier samples appeared unaltered. By the end of six months all samples showed good growth except for those at room temperature and those in water.

At the end of six months, samples stored in the refrigerator were split and transferred to a laboratory ultracold freezer at -80°C/-112°F and to liquid nitrogen at -196°C/-320°F. These latter conditions were only short-term to verify that such conditions would not fracture or otherwise harm the pollen. Samples previously maintained at humidities of 0-40%R.H. that were held at -196°C for 20 hours and -80°C for over a month showed no noticeable reduction in germination. At the end of 10 months storage at -18°C, pollen showed a great reduction in germination. Those dried before storage showed the most activity, but still only about 40% of the pollen was active. The other samples showed no germination, except for two samples that had been air dried to approximately 40%R.H. and stored at -18°C.

These latter two samples had 0.15 to 5% germination after 24 hours. The early death of this pollen may also have been influenced by the repeated freezing and thawing that samples underwent with multiple extractions of pollen samples for test and attempted pollinations. Drying and continuous ultracold conditions (-80°C and below) appear to be the best method for storing *Nepenthes* pollen for extended periods.

Nepenthes ventricosa pollen that had been frozen in liquid nitrogen (20 hours) and the remainder of six months at -18°C was used to pollinate a female *N. khasiana*. The resulting hybrid seed had a relatively good germination, but somewhat slow; the first dozen seedlings were only visible after three months.

Pollen intended for prolonged storage should be shipped on dry-ice, or minimally with ice packs in as fast a transport as possible. Because all *Nepenthes* are CITES listed (Appendix I and II), shipping rapidly between countries may be somewhat complicated.

Conservation Implications

The convention used in the past for greenhouse pollination of carnivorous plants (especially for production of hybrids in the genus *Nepenthes*) was to pollinate heavily and often. While this method ensures good seed set (if the pollen is viable) it requires more pollen than might be available for endangered plants. Poor quality pollen or incorrect placement of the pollen will result in the waste of receptive stigmas and pollen.

Optimal temperatures for growing lowland *Nepenthes* plants may be relatively high and constant (21-29°C/70-85°F), whereas those of the highland plants are lower with a nocturnal drop (10-21°C/50-70°F). As discussed above, pollen of *N. ventricosa* (an intermediate species kept in highland conditions) is very short lived when left on the plant. High humidity/moisture and light are likely to be important in limiting pollen viability. Pollen placed on a female stigma at the wrong time of day will be exposed to those deleterious conditions until the stigma is more active (perhaps the next night or day). In the wild, pollination of *Nepenthes* is thought to be primarily by insects because the flowers secrete nectar that is attractive to insects and the pollen is released in relatively large tetrads with echinate sculpturing (Kato, 1993, and citations therein). In order to achieve maximal seed set, healthy active pollen must be used when the stigma is receptive. Unless receptivity data for each species suggest otherwise, it is probably best to place pollen on stigmas surrounded by sepals that are secreting nectar and a scent; thus, mimicking insect pollinator activity. Experiments should be conducted to better define receptivity periods based on morphology of the sepals (erect versus reflexed) and nectar production so that growers can be prepared to attempt pollination during the most ideal conditions. Dafni & Motte Maués (1998) have developed a rapid and relatively simple procedure for determining stigma receptivity (involving detection of enzymatic activity). This

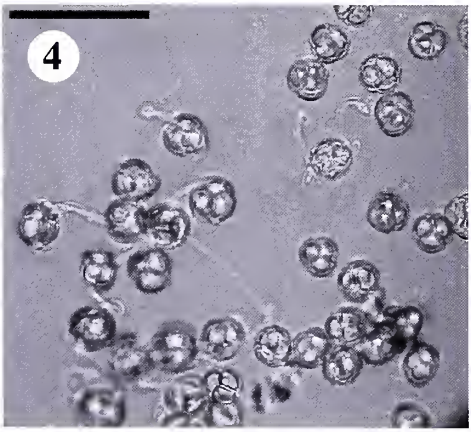


Figure 4: *Nepenthes ampullaria* pollen with tubes in 0.6 M sugar solution following storage at -80°C for six months. Scale bar = 0.1 mm.

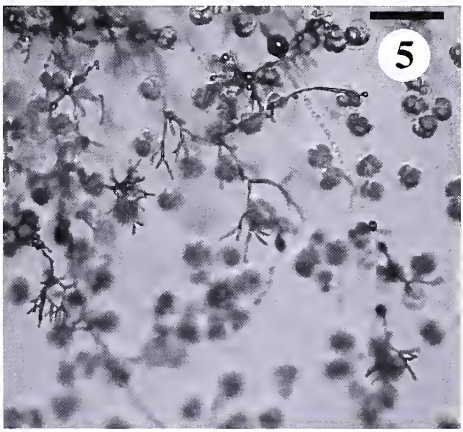


Figure 5: Contaminated sample showing multi-tipped fungal growth among *Nepenthes ampullaria* pollen grains. Scale bar = 0.1 mm.

method however is destructive to the stigmas tested.

Pollen should be collected from the entire inflorescence by tapping it over wax paper. This should be done at the beginning of any nectar/scent production (to remove old pollen) and at the end of the day/night period to collect the most active pollen. Some experimentation on collection will be needed with species that produce large amounts of nectar because the nectar tends to trap the pollen. If necessary, anthers covered in pollen can be detached with dissection tools and collected directly into tubes. Percentage of pollen activity or quality should be determined prior to any long-term storage, so that time, supplies, and effort will not be wasted on low quality or dead pollen. Because varying humidities below 40% did not appear to alter the germinability of the pollen that was stored for less than six months, but did for longer periods, it is suggested that pollen be dried with a desiccant. It is also much easier to obtain and maintain this low humidity outside of the laboratory and previous studies with pollens and seeds of other species found drier conditions best. Pollen should be stored at the lowest temperature available. Flash freezing and rapid thawing from liquid nitrogen does not appear to alter the viability of relatively dry *Nepenthes* pollen. If large quantities of pollen are collected (or collected over several days), it should be split into several tubes so that opening one tube will not require thawing of all the pollen. A portion of all male flowerings of endangered *Nepenthes* species should be banked at an institution capable of storage at -80°C or below. This pollen can then possibly be used years later if genetic contamination (hybrids) or a reduction in genetic diversity is discovered in the remaining plants.

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Sarracenia 'Melissa Mazur', P.Wilson, Carniv.Pl.Soc.J.(UK) 27:42 (2005), 14 Nov.
Sarracenia 'Victoria Morely', S.Morely, Carniv.Pl.Soc.J.(UK) 27:15 (2005),14 Nov.
Dionaea 'Green Dragon', M.Erbacher & M.Stoeckl, Taublatt 51:25 (2005), 24 Nov.
Dionaea 'Holland Red', M.Erbacher & M.Stoeckl, Taublatt 51:21 (2005), 24 Nov.
Dionaea 'Red Burgundy', M.Erbacher & M.Stoeckl, Taublatt 51:22 (2005), 24 Nov.
Utricularia 'Betty's Bay', S.Morely, Carniv.Pl.Soc.J.(UK) 27:33 (2005), 14 Nov.
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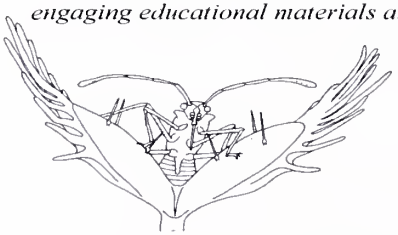

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